SCIENCE CHINA
Life Sciences

SPECIAL TOPIC: Plant biology: chromatin, small RNA and signaling
• REVIEW •

March 2015 Vol.58 No.3: 240–245
doi: 10.1007/s11427-015-4818-3

Recent advances in plant centromere biology

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Received November 10, 2014; accepted November 29, 2014; published online February 9, 2015

The centromere, which is one of the essential parts of a chromosome, controls kinetochore formation and chromosome segregation during mitosis and meiosis. While centromere function is conserved in eukaryotes, the centromeric DNA sequences evolve rapidly and have few similarities among species. The histone H3 variant CENH3 (CENP-A in human), which mostly exists in centromeric nucleosomes, is a universal active centromere mark in eukaryotes and plays an essential role in centromere identity determination. The relationship between centromeric DNA sequences and centromere identity determination is one of the intriguing questions in studying centromere formation. Due to the discoveries in the past decades, including "neocentromeres" and "centromere inactivation", it is now believed that the centromere identity is determined by epigenetic mechanisms. This review will present recent progress in plant centromere biology.

centromere, epigenetics, CENH3

Citation: Feng C, Liu YL, Su HD, Wang HF, Birchler J, Han FP. Recent advances in plant centromere biology. Sci China Life Sci, 2015, 58: 240−245, doi: 10.1007/s11427-015-4818-3

The centromere, which is one of the essential parts of a chromosome, controls kinetochore formation and chromosome segregation during mitosis and meiosis. Because of its complicated structure and sequence composition, the centromere was considered to be a great challenge to be studied. But due to investigations in recent decades, some characteristics of centromeres have been uncovered in both animals and plants. The structure of centromeres can be divided into three types, namely point, regional, and holocentric centromeres [1,2]. The centromeric DNA sequences evolve rapidly and have few similarities among closely related species. The budding yeast centromere is simplest with only 125 bp DNA sequence in length. Fission yeast has a more complex centromere, which occupies a region of about 10 kb [3]. In higher eukaryotes, centromeres are mainly composed of repetitive sequences. In humans, there are abundant alpha-satellite repeat sequences specifically located in the centromere region [4]. In plants, the centromeric sequences are mainly composed of tandem repeats and retrotransposons. The histone H3 variant, CENH3 (CENP-A in human), which replaces the canonical histone H3 specifically in centromeric nucleosomes, is an almost universal mark for functional centromeres in eukaryotes, and is also essential for kinetochore formation. An interesting aspect of centromeres is that its identity is determined by epigenetic mechanisms [1,5–8]. This was concluded by experimental data showing that centromeres could undergo inactivation in dicentric chromosomes and neocentromeres could be formed on noncentromeric DNA. The molecular mechanisms of the centromere inactivation and neocentromere formation are

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still mysteries. In this review, we will present recent research progress in plant centromere biology, and focus on the epigenetic determination of centromere identity.

1 Centromere structure and DNA sequence

Centromeres have various structures among species. In budding yeast (*Saccharomyces cerevisiae*), the centromere contains only one centromeric nucleosome [9]. In fission yeast (*Schizosaccharomyces pombe*), *Drosophila* and human, they occupy regions from 10 kb to several megabases long in the chromosomes. In *Caenorhabditis elegans*, the holocentromere is formed by point centromeres that extend over the whole chromosome [10,11]. In higher plants, the vast majority are regional centromeres that typically are in the megabase range. In higher plants, the centromeres were mainly composed of tandem repeats and retrotransposons, which are interspersed (Figure 1). Through genetic analysis and sequencing, a 180-base pair repeat sequence was found to be the main component in *Arabidopsis* centromeres [12]. This was confirmed by ChIP using antibody against the *Arabidopsis* centromeric histone CENH3 (called HTR12 in *Arabidopsis*) [13]. In rice, the centromere was demonstrated to be composed of CentO (a 155-bp satellite repeat sequence) and CRR (centromere retrotransposon of rice) [14]. In maize, by taking advantage of a set of maize-oat addition lines, the maize centromeric sequences were revealed. The centromere sequence composition was similar to that of rice. It is also composed of two types of sequences, CentC (centromere repeat C) and CRM (centromeric retrotransposon of maize) [6,15] (Figure 2A and B). Fiber FISH results showed that the amount of CentC varied among different chromosomes, and the length of the CentC/CRM sequence mixtures was from 300 to more than 2800 kb among the 10 chromosomes in maize [16]. Several studies revealed that CENH3 is loading on part of the centromeric DNA sequences, and histone H3 nucleosomes and CENH3 nucleosomes are interspersedly arranged in the centromere region [17–19]. The plant centromere core region shows high frequency of unequal homologous recombination through chromosomal exchange, and the result is frequent and extensive DNA rearrangements of centromere regions [20].

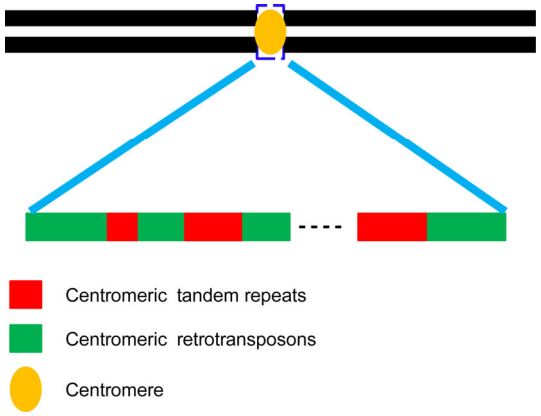

Figure 1 Model of plant centromeric sequence composition.

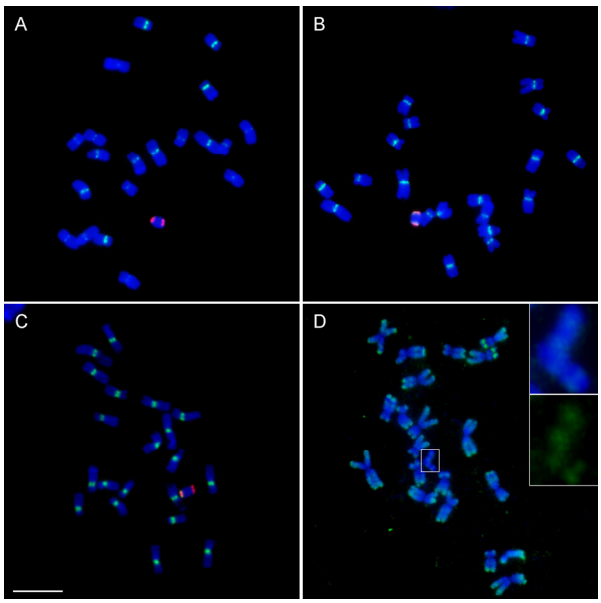

Figure 2 FISH (fluorescent *in situ* hybridization) and immunostaining of maize material containing a newly formed dicentric chromosome. CentC (green) and B-repeat (red) in (A), CRM (green) and B-repeat (red) in (B), anti-H2AThr133 antibodies (green) and B-repeat (red) in (C), anti-H4K12ac antibodies (green) in (D). Blue is DAPI. Scale bar, 10 μm.

2 Centromeric histone H3 variant CENH3

The histone variant CENH3 (CENP-A in human) is a universal functional centromere mark in eukaryotes. Though its function is conserved, the sequence of CENH3 evolves rapidly [21]. The paradoxical phenomenon was explained as a result of the adaptive evolution [22,23]. The core domain of CENH3 was called CENP-A targeting domain (CATD), which confers kinetochore formation and centromere targeting. CENH3 (CENP-A) was first identified in humans through biochemical studies [24]. Partial sequence analysis indicated that it was a new histone and essential for centromeres [25]. As CENH3 sequence is conserved among species, sequence could be gained by searching the database (BLAST) for species with genome or transcriptome data. CENH3s had been identified in a number of plant species. Two groups reported the characterization of CENH3 in *Arabidopsis thaliana* and maize in 2002, respectively [19,26].

Studies on the relationships between CENH3 protein structure and function were performed in several species, such as budding yeast (*S. cerevisiae*), *Drosophila melanogaster* cell lines and human cell lines. It was revealed that the core domain for centromere targeting was involved in the C terminal histone fold domain, and the detailed amino acid sequences corresponding to the function were different

among them [27−31]. In plants, similar work had been carried out in the model dicot plant *A. thaliana* [32]. By using a *cenh3* null mutant, the endogenous CENH3 could be completely replaced by transgenic protein variants [33]. Through substituting the CENH3 N terminal domain or C terminal domain with the histone H3.3, the results showed that either the H3 N terminal tail or the CENH3 N terminal tail combined with the CENH3 C terminal were sufficient for mitosis, while inserting the CATD domain of CENH3 into the H3 could not complement the function of CENH3 [32]. One transgenic protein variant referred to as *GFP-tailswap*, in which the CENH3 N terminal domain was substituted by the H3.3 N terminal domain with a GFP fluorescent protein in front of the fusion protein, could rescue the lethal phenotype of the *cenh3* null mutant but was highly sterile. Because the plants were not completely sterile, when crossed with wild plants, either as male or female, haploid progeny would be generated [33]. The results revealed that the centromere was important for chromosome inheritance. A different approach in barley also implicated cenh3 in haploid production [34]. After studying the meiotic phenotypes of the plants expressing the *GFP-tailswap* variant, the critical role of the CENH3 N terminal tail in meiosis was uncovered [35].

3 Centromere identity is epigenetically determined

With the elucidation of centromere DNA sequences in several species, it became clear that there were specific DNA sequences at the primary constriction. Does the sequence specificity of the centromere determine centromere formation? The most direct way to verify this question was to transform the centromere sequences into the organism from which they were derived, and see whether new centromeres could be formed on the exogenous centromeric sequences. Research in budding yeast gives positive results. The centromeric sequence of budding yeast was sufficient for functional centromere formation [9]. In human cell lines, through combining long alpha satellite arrays with telomere and other genomic DNA sequences, artificial minichromosomes could contain functional centromeres [36]. However, similar experiments showed different results in plants. Phan et al. [37] transformed rice with BACs containing maize or rice centromeric DNA sequences through particle bombardment, and as a control, BACs containing random rice genomic DNA sequences. Though the exogenous centromeric DNA sequences could be integrated into the rice genome and transmitted to the next generation, no functional centromere could be formed. The results indicated that in plants, the centromeric DNA sequence alone is insufficient to induce the formation of a centromere.

While centromeric DNA sequences were insufficient to *de novo* form a functional centromere, is it necessary for functional centromere formation? The answer is negative. The evidence is derived from the finding of centromere formation at chromosomal sites where no centromeric DNA sequences exist—a situation referred to as neocentromeres. In human and *Drosophila*, neocentromeres had been reported to be formed on several genomic regions [38,39]. In plants, the first case of the neocentromere was reported in barley. Using a gametocidal system, two telosomes lacking both the barley centromeric satellite sequences and wheat centromeric tandem repeats were generated. Although there was no centromeric sequence on these two chromosomes, they transmitted normally and several functional centromere specific proteins could be detected by immunostaining with specific antibodies, so the results indicated that neocentromeres had been formed on these two chromosomes [40]. In an oat-maize addition line, a neocentromere formed on maize chromosome 3 in an oat background. Due to chromosome breakage, a fragment of the short arm which lacked normal centromeric DNA sequences CentC and CRM (centromere retrotransposon of maize), still could transmit to the offspring. The oat CENH3 protein signals could be detected on the fragment by immunostaining [41]. In maize, one case was a minichromosome derived from chromosome 3, named Duplication 3a (Dp3a), generated by UV-irradiation. The main maize centromeric DNA sequences, CentC and CRM, could not be detected on this minichromosome, but it could be transmitted. Immunostaining experiments showed that the functional centromere specific proteins were present on the minichromosome. These results indicated the formation of a neocentromere on this minichromosome. Further, ChIP (chromatin immunoprecipitation)-seq data showed that the neocentromere was located over a 350 kb region [42]. Another example was a newly formed B chromosome-centromere, which lacked maize centromeric sequence CentC and contained an extremely low content of CRM and B-repeat (B chromosome specific repeat) sequences. Immunostaining and other analysis also revealed the formation of a neocentromere, which was located on an 723 kb region of chromosome 9S [43]. Taken together, the results revealed that centromeric DNA sequences are not necessary for centromere formation.

The phenomenon of centromere inactivation also provided other evidence that centromere identity was epigenetically determined. A translocation between the maize B chromosome and chromosome 9 generated TB-9Sb. The chromosome included the B centromere and part of chromosome arm 9S. After adding a reverse duplicated 9S, the modified chromosome B9-Dp9 was produced. Because this chromosome had two copies of 9S in an opposite direction, during meiosis, dicentric chromosome would be formed through intra-chromosome recombination. As a chromosome with two active centromeres would be unstable, the chromosome would break in anaphaseII, and trigger the BFB (breakage-fusion-bridge) cycle [44]. A number of minichromosomes could be recovered in the progeny. By

using centromere repeat sequences as probes to analyze the minichromosomes by FISH (fluorescent *in situ* hybridization), the results showed that in some of the minichromosomes, there were two B centromeric regions at the sequence level. However, when immunostaining experiments were performed, the functional centromere marks such as CENH3 and CENPC could only be detected on one centromere region. Thus, the other centromere was inactivated [45]. Interestingly, when these dicentric chromosomes broke into two parts, each with one centromeric region, the inactive centromere could regain activity [46]. As another example, a translocation formed between maize chromosomes 1 and 5 captured a centromere that became inactive showing no CENPC and H3S10ph staining, which is otherwise typical of active centromeres [47,48]. Centromere inactivation also had been reported in wheat [49].

At present the evidence suggests that centromere identity is determined by epigenetic mechanisms [1,7,50]. However, how it is controlled and regulated in this way is still elusive. Studies of CENH3 indicate that it plays a critical role in centromere identity. But the molecular mechanisms of centromere inactivation and neocentromere formation are still mysteries. The role that post-translational histone modifications play in centromere identity determination has also been explored. In *Drosophila melanogaster* and human cell lines, research on histone modifications in the centromeric chromatin region revealed that the pattern of histone modifications is distinct from other regions of the chromosome arms [51]. In wheat, centromere inactivation resulted in elevated H3K27me2 and H3K27me3 concentrations [49]. Another histone modification mark, namely H2A-Thr133, was also reported as a characteristic of active centromeres (Figure 2C), but the detailed relationship between this histone modification and centromere function is still unknown [52]. The histone modifications of maize dicentric chromosomes had been studied. There are differences between the active and inactive centromeres (unpublished data) (Figure 2D). The aforementioned data provide evidence that there were relationships between centromere identity and centromeric histone modifications. However, the detailed connection is unknown. By applying a DNA fiber-based technique, compared to the active B centromere in maize, the inactive B centromere exhibits DNA hypermethylation [53]. Another epigenetic regulatory factor is non-coding RNA. The non-coding RNA has also been reported to take part in the centromere functioning in several studies [54–58]. Recently, it was reported that maize centromeres would adopt a larger size in an oat background [59]. Taken together, it seems that the centromere identity is determined by multiple processes (Figure 3).

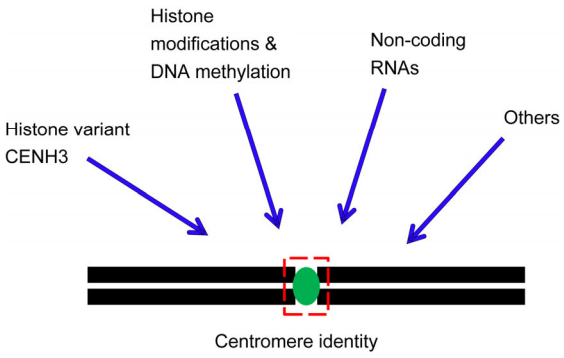

Figure 3 Putative epigenetic mechanisms for centromere identity determination. Black lines represent chromosome arms and green ellipse represents the centromere.

4 Conclusion

In recent decades, striking progress has been made in plant centromere biology, including the study of centromere structure, DNA sequences, function, genomics, and more recently, centromere function in homologous chromosome pairing [60–62]. However, there are still several pivotal questions to be answered. First, do the centromeric DNA sequences play a role in centromere function? Second, how is centromere identity determined by epigenetic mechanisms? Third, what is the role of non-coding RNAs in a centromere? Research on plant centromere biology in the past and the future will contribute to plant genetic engineering (especially plant chromosome engineering), ultimately benefiting plant breeding.

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